Supporting Information

An anisotropic hydrogel based on mussel-inspired conductive ferrofluid composed of electromagnetic nanohybrids

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1. Preparation of the hydrogels

**Materials used:** Dopamine (DA), acrylamide (AM), ammonium persulfate (APS), N,N’-Methylene bisacrylamide (MBA), tetramethylethylenediamine (TMEDA), ferric chloride (FeCl$_3$·6H$_2$O), ferrous chloride (FeCl$_2$·4H$_2$O), and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (USA). Carbon nanotubes (CNTs, diameter: 30–50 nm, length: 10–20 μm) were purchased from Beijing DK Nano technology Co., LTD China. All chemicals were reagent grade.

1.1 Synthesis of PDA-CNT-Fe$_3$O$_4$ (PFeCNT) nanohybrids

The PDA-CNT-Fe$_3$O$_4$ (PFeCNT) nanohybrids were prepared by a polydopamine (PDA)-mediated precipitation method. First, 0.2 g of DA was added to a three-necked flask containing 12.5 ml deionized water, and then 2 g of CNT was dispersed into the DA solution and stirred for 30 min at room temperature to allow DA coating on the surfaces of CNT. Second, 2 g of FeCl$_2$·4H$_2$O and 5.2 g of FeCl$_3$·6H$_2$O were dissolved into the DA-CNT dispersion and stirred for another 10 min. Third, HCl (4.25 M, 0.85 ml) was added into this mixture solution to ensure the balance of Fe (II) and Fe (III) ions. Fourth, the three-necked flask was heated to 100°C in a water bath. Fifth, NaOH solution (1.5 M, 250 ml) was added dropwise to the mixture solution rotating at 700 rpm and stirred for 1 h to precipitate and coat the surfaces of CNTs with magnetite Fe$_3$O$_4$ nanoparticles (NPs). The precipitate was collected using a magnet to remove the uncoated-CNT. The collected precipitates was further centrifuged at 4000 rpm for 5 min to collect PDA-CNTs-Fe$_3$O$_4$ (PFeCNT) nanohybrids, while the bare Fe$_3$O$_4$ NPs were discarded. Finally, the PFeCNT nanohybrids were washed with deionized water until the pH of the suspension became to neutral, and the nanohybrids’ aqueous dispersion was lyophilized in vacuum at -80°C to obtain the purified PDA-CNTs-Fe$_3$O$_4$ (PFeCNT) nanohybrids. The CNTs-Fe$_3$O$_4$ (FeCNT) complex without adding DA was prepared by the same method. The PDA-Fe$_3$O$_4$ nanoparticles (PFe NPs) without adding CNTs were also prepared by the same method to demonstrate the controlling effect of PDA on the formation of magnetic crystals. The bare Fe$_3$O$_4$ nanoparticles (Fe$_3$O$_4$ NPs) without adding CNTs and DA were prepared as controls.
The weight percentage of iron oxide nanoparticle in the PFeCNT nanohybrids was measured by dissolving the iron oxides in hydrochloric acid solution. Briefly, 0.5 g PFeCNT nanohybrids were soaked in HCl solution (10 ml, 10 wt.%). The control samples (pure Fe₃O₄ nanoparticles) were also soaked in HCl solution with the same concentration. After 1 week of soaking at room temperature, the un-dissolved CNTs in the solution were collected after centrifugation (12 000 g for 10 min), and vacuum dried at 60 °C, then weighed.

**Table S1** Formula compositions of nanomaterials

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial weight /g</th>
<th>Remanent weight /g</th>
<th>Weight ratio of CNT (%)</th>
<th>Weight ratio of iron oxide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₃O₄ nanoparticles</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>PFeCNT nanohybrids</td>
<td>0.5</td>
<td>0.27</td>
<td>54</td>
<td>46</td>
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</table>
1.2 Fabrication of the isotropic and anisotropic PFeCNT hydrogels

The PDA-CNT-Fe₃O₄ (PFeCNT) nanohybrids incorporated anisotropic hydrogels were formed by free-radical polymerization of AM with the assistance of oriented magnetic field. First, the PFeCNT nanohybrids were dispersed into a pre-polymerized PDA (1 mg/ml) solution to form the PFeCNT based ferrofluid by using PDA as the dispersed medium. Second, AM monomers, ammonium persulfate (APS), N,N-methylenebis (acrylamide) (MBA), and tetramethy-llethlyenediamine (TMEDA) were added to the PFeCNT-ferrofluid in an ice bath while stirring. After stirring for 5 min, the ice bath and stirrer were removed, and the obtained mixture was injected into molds with and without being subjected to a magnetic field (Scheme S1). A customized static magnetic coil was used to provide magnetic flux. The coil was connected to a 15 V DC power source and the field generated was proportional to the electric current (DC) passing through the coil. The magnetic field strength was measured experimentally using a GM08 gauss meter (Hirst Magnetic Instruments Ltd, USA). After the alignment of the nanohybrids was completed by the magnetic flux, the AM monomers were polymerized and crosslinked to form the hydrogels in the presence of APS, MBA, and TMEDA. CNT-Fe₃O₄ (FeCNT) complex incorporated hydrogels were also prepared with and without 30 mT magnetic field as a control. Pure polyacrylamide (PAM) hydrogels were also prepared as control. The compositions of the various hydrogels are listed in Table S2.

Scheme S1. Schematics of the anisotropic and isotropic hydrogels in the presence or absence of magnetic field.
<table>
<thead>
<tr>
<th>Hydrogels</th>
<th>Nanohybrids / AM (wt. %)</th>
<th>DA/AM (wt. %)</th>
<th>AM (g)</th>
<th>APS/ AM (wt. %)</th>
<th>MBA/ AM (wt. %)</th>
<th>TMEDA (μl)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAM hydrogel</td>
<td>0</td>
<td>0</td>
<td>2.6</td>
<td>0.5</td>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>2.6</td>
<td>0.5</td>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>FeCNT hydrogels</td>
<td>10</td>
<td>0</td>
<td>2.6</td>
<td>0.5</td>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0</td>
<td>2.6</td>
<td>0.5</td>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>PFeCNT hydrogels</td>
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<td>2.6</td>
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</table>
2. Characterizations

2.1 Characterization of PDA-CNT-Fe\textsubscript{3}O\textsubscript{4} nanohybrids

The crystalline phase of the PDA-CNT-Fe\textsubscript{3}O\textsubscript{4} (PFeCNT) nanohybrids, CNT-Fe\textsubscript{3}O\textsubscript{4} (FeCNT) complex, PDA-Fe\textsubscript{3}O\textsubscript{4} (PFe\textsubscript{3}O\textsubscript{4}) NPs, and bare Fe\textsubscript{3}O\textsubscript{4} NPs was identified by X-ray diffraction (XRD, X’pert PRO, Philips, The Netherlands) by operating at a voltage of 40 kV with a current of 40 mA, and employing Cu Kαfiltered radiation (λ = 1.5406 nm).

The morphologies of PFeCNT nanohybrids, FeCNT complex, PFe\textsubscript{3}O\textsubscript{4} NPs, and Fe\textsubscript{3}O\textsubscript{4} NPs were examined with a scanning electron microscope (SEM, JSM 6390, JEOL, Japan) and transmission electron microscopy (TEM, JEM-2010, JEOL, Japan).

The PDA decorated CNTs (PDA-CNTs) were characterized using an X-ray photoelectron spectrometer (XPS, Kratos, Axis Ultra DLD, UK). A monochromatic Al Kα X-ray was used as an excitation source (hv = 1486.6 eV) running at 15 kV and 150 W. The morphology of PDA-CNTs was observed by a transmission electron microscope (TEM, JEOL, JEM-2010F, Japan). The samples were prepared by dropping the prepared aqueous PDA-CNTs dispersion onto the amorphous carbon-coated copper grids and dried.

The magnetizations (M) of the PFeCNT nanohybrids, PFeCNT complex, PFe\textsubscript{3}O\textsubscript{4} NPs, and Fe\textsubscript{3}O\textsubscript{4} NPs were characterized as a function of the applied field (H) at 293 K by using a vibrating sample magnetometer (VSM, LakeShore, 7037, USA).

2.2 The microstructures of the dried PFeCNT-PAM hydrogels

The microstructures of the dried PFeCNT-PAM hydrogels were examined using the SEM (JSM 6390). Before examination, the hydrogels were freeze-dried, and cut to expose their inner cross-section, and sputtered with gold.

2.3 Characterization of mechanical properties of the hydrogels

The anisotropic mechanical behaviors of the hydrogels were tested in directions parallel (∥) and perpendicular (∥) to the alignment of the PFeCNT nanohybrids (Scheme S2). The isotropic hydrogels with disordered PFeCNT nanohybrids were also tested as controls. The
tensile strength and compressive strength of the hydrogels were measured by using the as-prepared samples before swelling.

**Scheme S2.** (a) Illustration of the mechanical testing on anisotropic and isotropic hydrogels. The external force was parallel to the oriented nanohybrids. The external force was perpendicular to oriented nanohybrids. The isotropic hydrogel with random nanohybrids. (b) Illustration of the fracture energy test. When the force was parallel to the oriented nanohybrids, the aligned nanohybrids can prevent crack propagation. When the force was perpendicular to the oriented nanohybrids, the crack is easily to propagate.

### 2.3.1 Tensile tests

For this test, the hydrogels with a strip shape (25 mm × 25 mm × 2 mm) were prepared. Then the hydrogels were conducted using a universal test machine (UST, Instron 5567, USA) with a 100 N load cell. The gauge length between the clamps was 5 mm. The loading rate was 100 mm/min. The tensile strength ($\sigma_T$) was calculated as maximum tensile load divided by cross-sectional area. The extension ratio (ER, $\lambda$) was defined as the extended length relative to the original length.

### 2.3.2 Determination of fracture energy

The fracture energy ($G$) of the hydrogels was tested by the single edge notch test on the universal test machine (Instron 5567), as reported in our previous study.

### 2.3.3 Compressive tests

The hydrogels with a cube-shape (10 mm × 10 mm × 10 mm) were prepared. Then the hydrogels were compressed using the UST (Instron 5567) with a 2 kN
load cell. During compression, the speed of cross-head was 5 mm/min. The compressive strength ($\sigma_C$) was defined as the stress when the compress strain reached to 80%.

2.4 Conductivity measurement of the hydrogels.

The conductivity of the hydrogels was measured by a two-probe method using a potential state (CHI 660, USA). The conductivity ($\kappa$, S/cm) was calculated according to the Equation 1.

$$\kappa = \frac{I}{V} \frac{L}{A}$$  \hspace{1cm} \text{(Equation 1)}

where $V$ (V) is the measured voltage; $I$ (A) is the current provided by the potential state; $A$ (cm$^2$) is the cross-sectional area of the sample, and $L$ (cm) is the distance between the two probes.

The conductivity of the hydrogel was tested along different directions (Scheme S3).

$\kappa_{||}$ denotes the conductivity measured along the direction parallel to the alignment of the nanohybrids, and $\kappa_{\perp}$ denotes the conductivity measured along the direction perpendicular to the alignment of the nanohybrids. The anisotropic degree of conductivity was calculated as $\kappa_i = \frac{\kappa_{||}}{\kappa_{\perp}}$.

The conductivity of the isotropic hydrogel with disordered PFeCNT nanohybrids was also measured.

Scheme S3. Illustration of the conductivity testing on anisotropic and isotropic hydrogels.

2.5 Tissue adhesion tests of the hydrogels.
A tensile-adhesion test was used to evaluate the adhesiveness of the hydrogels on various substrates, including glass, polytetrafluoroethylene- (PTFE), and titanium-based substrates. Porcine skin was used as the representative tissue. Three kinds of hydrogels were tested, including pure polyacrylamide (PAM) hydrogels, FeCNT complex incorporated hydrogels (FeCNT-PAM hydrogels), and PDA-CNT-Fe$_3$O$_4$ nanohybrids incorporated hydrogels (PFeCNT-PAM hydrogels) with different content of PFeCNT nanohybrids (0, 5, 10, 15 wt.%) by using porcine skin as the representative substrates. The hydrogels were first applied to the surface of the substrates with a bonded area of 25 mm × 20 mm (Scheme S4). The samples were then pulled to failure by the UTM (Instron 5567) with a cross-head speed of 0.5 mm/min under the ambient conditions. The adhesion strength was calculated by measuring maximum load divided by the bonded area.

Scheme S4. Schematics of adhesion tests.

2.6 Statistical analysis

The data were analyzed by one-way analysis of variance followed by Tukey multiple-comparison post hoc test to determine significant difference between test groups. The level of statistical significance was set to p < 0.05.
3. Results

3.1 Electric conductivity of the PDA-based ferrofluid

The electric conductivity of the PDA-based ferrofluid was tested by using a conductivity probe (Model AZ86505), according to previous study.\textsuperscript{1}

As shown in Figure S1, DI water is insulating (0.003 mS/cm), and the alginate solution-based ferrofluid has a poor electric conductivity of 1.72 mS/cm. In contrast, PDA-based ferrofluid has a high conductivity of 77.67 mS/cm. This is because PDA is a hybrid electronic-ionic conductive material, and therefore the PDA solution is a good medium for the preparation of a conductive ferrofluid.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{Figure_S1.png}
\caption{Electric conductivity of different solution. The concentration of PFeCNT nanohybrids in each solution is 10 wt.\%.
}
\end{figure}
3.2 PDA coatings on the surfaces of CNTs

Figure S2. (a) TEM image of PDA decorated CNTs. (b, c) XPS spectra of the CNTs before and after PDA decoration.

TEM image showed that the outer surfaces of the CNTs grew a continuous and smooth membrane with amorphous structures, indicating that the PDA film covered on the CNT (Figure S2a). As shown in Figure S2c, the peaks of XPS spectra of N1s at 400 eV and 403.1 eV correspond to -NH$_2$, C=N(H)-C species, respectively, which were attributed to specific groups of PDA. These results indicated that PDA was successfully decorated on the surfaces of CNTs after polymerizing dopamine in water under alkali environment. 2
3.3 PDA mediating the formation of PFe$_3$O$_4$ NPs

Figure S3. (a) SEM images, (b) XRD patterns, and (c) magnetization curves of various Fe$_3$O$_4$ nanoparticles synthesized with a series of dopamine (DA) concentrations. (d) FT-IR spectra of Fe$_3$O$_4$ and PFe$_3$O$_4$ NPs.

The morphology of Fe$_3$O$_4$ NPs synthesized under different conditions is presented in Figure S3a. From SEM images, we can see that pure Fe$_3$O$_4$ NPs has globular shapes. It is interesting to note that the PDA-controlled Fe$_3$O$_4$ NPs have smaller size than pure Fe$_3$O$_4$ NPs. Figure S3b shows the XRD patterns of Fe$_3$O$_4$ particles synthesized at various DA concentrations, which corresponded well to magnetite Fe$_3$O$_4$. In addition, the intensities of the diffraction peaks of the PDA-mediated nanoparticles were slightly weaker than that of the pure Fe$_3$O$_4$ NPs, which indicated that the surface-active PDA tended to hinder the growth of the nanoparticles. The magnetization curves of the synthesized Fe$_3$O$_4$ NPs are presented in Figure S3c. The curves did not show any hysteresis behavior for all samples, and exhibited immeasurable values of coercivity field and remnant magnetization, indicating that the synthesized particles possessed superparamagnetic properties. The saturation magnetization ($M_s$) decreased with the increase of DA concentration. In summary, the results of XRD, SEM, and magnetic analysis give the formative evidence that DA can control the nucleation and growth of the magnetite nanoparticles.
3.4 Microstructure of the PFeCNT-PAM hydrogel

Figure S4. (a) SEM image of the cross-section of PFeCNT-PAM hydrogel (10 wt.%). (b) Magnified image showed that CNTs were well integrated with matrix. (c) EDS mapping.

Results: The SEM micrograph of cross-section of the hydrogel containing 10 wt.% PFeCNT nanohybrids is shown in Figure S4. The PFeCNT nanohybrids were uniformly distributed in the hydrogel and integrated with the hydrogel matrix. This was attributed to the reactive catechol groups on the PFeCNT, which stabilized the nanohybrids dispersion by strong interactions with the PAM chains, and led to the well integration between the PFeCNT and the polymer network.\textsuperscript{5,6} This result indicated that PDA-based functionalization was a potent method to take full advantage of the remarkable physical and mechanical properties of CNTs.
3.5 CLSM micrographs of the hydrogels with different contents of PFeCNT nanohybrids

Figure S5. CLSM micrographs of the anisotropic hydrogels with different contents of PFeCNT nanohybrids: (a) 5 wt.%, (b) 10 wt.%, and (c) 15 wt.%. 
3.6 Optical behavior of the anisotropic and isotropic hydrogels

A 650 nm mode laser was used as the incoming light source. A camera was used to record the scattered light distribution in the 2D plane perpendicular to the light propagation direction (Figure 3e), and then the light intensity was measured in varied scattering gap along the x and y directions.

Figure S6. Optical images of (a) the anisotropic hydrogels incorporated with aligned PFeCNT nanohybrids, (b) the isotropic hydrogels incorporated with randomly distributed PFeCNT nanohybrids, and (c) the isotropic hydrogels incorporated with randomly distributed FeCNT nanohybrids. (d) The aligned PFeCNT-hydrogel showed anisotropic light scattering. (e) The random-PFeCNT hydrogel showed isotropic light scattering. (f) FeCNT incorporated hydrogel showed isotropic light scattering, because the CNT did not align in the hydrogel under magnetic field.
### 3.7 Anisotropic degree

**Table S3** Anisotropic degree of the anisotropic hydrogels in terms of different properties.

<table>
<thead>
<tr>
<th></th>
<th>PFeCNT contents in the hydrogel (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Extension ratio ($\lambda_///\lambda_{\perp}$)</td>
<td>1.22</td>
</tr>
<tr>
<td>Tensile strength ($\sigma_{T//}/\sigma_{T_{\perp}}$)</td>
<td>1.48</td>
</tr>
<tr>
<td>Compressive strength ($\sigma_{c//}/\sigma_{c_{\perp}}$)</td>
<td>1.45</td>
</tr>
<tr>
<td>Fracture energy ($G_{//}/G_{\perp}$)</td>
<td>1.47</td>
</tr>
<tr>
<td>Conductivity ($\kappa_{//}/\kappa_{\perp}$)</td>
<td>1.42</td>
</tr>
</tbody>
</table>
3.8 Adhesiveness of PFeCNT-PAM hydrogels

Figure S7. The adhesive strength of the hydrogels with different PFeCNT contents to porcine skin. Error bar represents the standard deviation of five samples.
3.9 Effect of the magnetic field strength on the properties of hydrogels

The external magnetic field had an impact on the orientation of the PFeCNT nanohybrids, and thus affected the anisotropic properties of the hydrogels. Higher magnetic field (50 mT) led to the aggregation of nanohybrids at the edges of the sample. While lower magnetic field (10 mT) could not control the orientation of nanohybrids in the hydrogel and therefore the hydrogels were isotropic (Figure S8). Thus, the optimal strength of magnetic field required for the preparation of anisotropic hydrogels was 30 mT.

![Figure S8](image)

**Figure S8.** (a) Optical images of the PFeCNT hydrogels formed under different magnetic fields. Effect of the magnetic strength on the properties of the hydrogels, including (b) extension ratio, (c) tensile strength, (d) compressive strength, and (e) conductivity. The hydrogels contained 10 wt.% of PFeCNT nanohybrids. All of these properties were measured along the alignment of PFeCNT nanohybrids in the hydrogels.
3.10 Comparison of the PFeCNT-PAM hydrogels with previously reported CNT-incorporated hydrogels

Figure S9. Comparison of the mechanical properties and conductivity of PFeCNT-PAM hydrogel reported in this work with those of previously reported CNTs-incorporated hydrogels.8-17 (a) Comparison of the conductivity, tensile strength, and extension ratio of different hydrogels. (b) Comparison of the conductivity and compressive strength of different hydrogels.

The conductivity of the PFeCNT-PAM hydrogel was as high as 0.6 S/m in parallel direction, which was comparable to that of previously reported CNT-incorporated hydrogels (Figure S9a). Meanwhile, the hydrogel showed high stretchability (1600%), which was superior to previously reported CNT-hydrogels.8, 9, 12, 13 Figure S9b compared the compressive strength and conductivity of CNT incorporated-hydrogels reported in the literature with those of our PFeCNT-PAM hydrogel. It can be seen that the PFeCNT-PAM hydrogel with the high compressive strength of 0.4 MPa was more robust than previously reported CNTs-incorporated hydrogels. This was because PDA on the PFeCNT ensured the well integration of the nanohybrids with polymer network. In short, this anisotropic hydrogel has great potential in a variety of applications that require the hydrogels simultaneously possessing high mechanical properties and conductivity.
3.11 Demonstration of the universality of the hydrogel preparation method

To demonstrate the universality of preparation method for the anistriopic hydrogel, we replaced AM monomers with acrylic acid (AA) monomers during gelation process to obtain PFeCNT-incorporated poly(acrylic acid) hydrogels (PFeCNT-PAA) under a directional magnetic field. The compositions of the PFeCNT-PAA hydrogels were listed in Table S4. The orientation and spatial distribution of PFeCNT nanohybrids in the hydrogel were observed by the CLSM microscopy. The mechanical behaviors and conductivity of the hydrogels were tested in directions parallel (/\) and perpendicular (⊥) to the alignment of the PFeCNT nanohybrids.

Table S4. Compositions of the hydrogel.

<table>
<thead>
<tr>
<th>Hydrogels</th>
<th>Nanohybrids /AA (wt. %)</th>
<th>DA/AA (wt.%</th>
<th>AA (g)</th>
<th>APS/ AA (wt.%</th>
<th>MBA/ AA (wt.%</th>
<th>TMEDA (μl)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFeCNT-PA A</td>
<td>10</td>
<td>0.4</td>
<td>2.6</td>
<td>0.5</td>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>

![Image a](image1.png)  
![Image b](image2.png)

![Image c](image3.png)  
![Image d](image4.png)
Figure S10. (a) CLSM micrograph of the anisotropic PFeCNT-PAA hydrogel with magnetically aligned PFeCNT. (b) Typical tensile and (c) compressive stress-strain curves of PFeCNT-PAA hydrogels, which were obtained by applying load in different directions (parallel or perpendicular to the alignment of PFeCNT nanohybrids). (d) Anisotropic conductivity of the hydrogel. The hydrogels contained 10 wt.% of PFeCNT nanohybrids.

As demonstrated by the CLSM micrograph (Figure S10a), the PFeCNT nanohybrids were well aligned in the hydrogel. As expected, the PFeCNT-PAA hydrogels also exhibited anisotropic mechanical properties and conductivity because the aligned PFeCNT nanohybrids reinforced the hydrogels in an anisotropic manner (Figure S10b, c, d). These results indicated that this study developed a universal strategy that could be used to prepare various anisotropic composites with different components.
3.12 The cytotoxicity assay of PFeCNT nanohybrids

In brief, cells with density of $2.5 \times 10^4$/sample were seeded in 96-well tissue culture plates. After 24 h of incubation at 37 °C, the medium was removed, and PFeCNT nanohybrids with a series of concentrations (0, 0.05, 0.1, 0.25, 0.5 mg/ml) in complete culture medium were added. Control wells contained culture medium alone. Cell proliferation was evaluated at 24 and 48 hours by MTT assay. 10 μL of 5 wt.% MTT contained medium was added to each well, and the plates were incubated at 37°C for 4 hours. After incubation, plates were read using a microplate reader (Model MQX200, BioTek, Germany) at 570 nm. As shown in Figure S11, no significant signs of toxicity were observed at PFeCNT nanohybrids concentration up to 0.1 mg/ml, which proved that the PFeCNT nanohybrids are cytocompatible.

![Figure S11. Proliferation of BMSCs exposed to a series of PFeCNT nanohybrid dispersion (0–0.5 mg/ml).](image-url)
3.13 Electrical stimulation of C2C12 cultured on the hydrogels

Before cell culture, the hydrogels (diameter: 8 mm, thicknesses: 2.5 mm) were first purified by deionized water thrice, sterilized by 75% alcohol for 24 h, and then immersed in sterilized PBS to remove any residual alcohol.

The anisotropic hydrogels with orientated PFeCNT nanohybrids (10 wt.%) were tested to evaluate the guidance effect of the hydrogels on cells under external electrical stimulation. The isotropic hydrogels with randomly distributed PFeCNT nanohybrids were set as controls. C2C12 myoblasts (Stem Cell Bank, Chinese Academy of Sciences) were used for electrical stimulation experiment. The C2C12 cells in passage 3 were seeded on the hydrogels with a density of $5 \times 10^5$ cells per sample. They were then cultured in DMEM (HyClone, USA), supplied with 10% fetal bovine serum (HyClone), and 1% penicillin-streptomycin solution (HyClone) in a CO$_2$ incubator at 37 $^\circ$C. After 1 day of culturing, the cells were stimulated with a series of electrical stimulation (ES) voltages supplied by a homemade high-throughput ES device (Figure S12).$^{21}$ This device is a 48-well culture plate with a cover, which was connected to a circuit by several titanium wires. In the plate, each well has two titanium (Ti) wires, working as the electrode. After switching on the external power supply, the voltage was applied to stimulate the cells on the films through these two Ti electrodes. ES voltages (0 mV, 300 mV, and 900 mV) were applied on the cells for 30 min per day, according to our previous studies.$^{19,20}$

![Figure S12. Homemade high-throughput ES device.](image)
After 1 and 3 days of stimulation, the morphology of the cells cultured on the hydrogels was observed by a confocal laser scanning microscope (CLSM TCSSP5, Leica, Germany) after the cells were stained by calcein AM (A017, USA). The proliferation of the cells was tested by an MTT assay [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, USA]. In order to further understand the behaviors of cells on the hydrogels after 3 days of electrical stimulation, the focal adhesion protein (vinculin) and nucleus was immunostained with focal adhesion staining kit and DAPI, respectively. Briefly, the samples were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA for 1 h. The samples were then incubated overnight at 4°C with ab196454 (Abcam). The nuclear of cells was stained with DAPI (Sigma, USA) for 10 min in the dark (shown in blue). Finally, the vinculin and nucleus of cells were observed by CLSM (Leica, Germany) and the aspect ratio of nucleus was calculated by referring to the previous literature. The aspect ratio of nucleus is defined as the ratio of the length of the major axis (the maximum distance between two points on the perimeter) and the minor axis (minimum distance between two points linked by a line crossing the center of mass of the particle).
Figure S13. Fluorescence micrographs of C2C12 cells cultured on anisotropic and isotropic hydrogels under different electrical stimulation voltage after 1-day culture. Scale bar: 100 μm
We quantified the intensity of the fluorescence antibody to represent the size of the focal adhesion from CLSM images. For quantification analysis, at least 10 staining images for each group were analyzed. The intensity in Figure S14 was the mean vinculin (VCL) intensity in regions of interest (ROIs) from more than 50 cells. Without electrical stimulation, the geometry cues of the anisotropic hydrogel can enhance vinculin accumulation, compared with the isotropic hydrogels. Under the electrical stimulation, significant increase in vinculin intensity was observed, indicating that C2C12 cells displayed larger focal adhesions on the anisotropic hydrogels.

**Figure S14.** The mean vinculin (VCL) intensity, normalized to the mean intensity determined under the “isotropic hydrogel + 0 mV” condition.
Figure S15. High-magnification CLSM micrographs of C2C12 cells on the anisotropic and isotropic hydrogels after 3 days of culturing under different electrical stimulation voltages. White arrowheads mark the sites of focal adhesions, which was further magnified in the right column. Scale bar indicates 5 μm.
References:


(4) Mascolo, M.; Pei, Y.; Ring, T. Room temperature co-precipitation synthesis of magnetite nanoparticles in a large pH window with different bases. *Materials* 2013, 6, 5549-5567.


